

EXPRESS MAIL NO.: EV 335 857 168 US

**INTRADERMAL DELIVERY OF VACCINES AND GENE THERAPEUTIC
AGENTS VIA MICROCANNULA**

[0001] This application is a continuation-in-part of application no. 10/185,717, filed July 1, 2002, which is a continuation-in-part of application no. 10/044,504 filed January 10, 2002, which is a continuation-in-part of applications no. 09/834,438 and 09/835,243, filed April 13, 2001, which are continuations-in-part of application no. 09/417,671, filed October 10, 1999 and claims priority to U.S. provisional application no. 60/301,476, filed June 29, 2001, each of which is incorporated herein by reference in its entirety.

1. BACKGROUND OF THE INVENTION

1.1 FIELD OF THE INVENTION

[0002] The present invention relates to methods and devices for administration of vaccines and gene therapeutic agents into the intradermal layer of skin.

1.2 BACKGROUND INFORMATION

[0003] The importance of efficiently and safely administering pharmaceutical substances for the purpose of prophylaxis, diagnosis or treatment has long been recognized. The use of conventional needles has long provided one approach for delivering pharmaceutical substances to humans and animals by administration through the skin. Considerable effort has been made to achieve reproducible and efficacious delivery through the skin while improving the ease of injection and reducing patient apprehension and/or pain associated with conventional needles. Furthermore, certain delivery systems eliminate needles entirely, and rely upon chemical mediators or external driving forces such as iontophoretic currents or electroporation or thermal poration or sonophoresis to breach the stratum corneum, the outermost layer of the skin, and deliver substances through the surface of the skin. However, such delivery systems do not reproducibly breach the skin barriers or deliver the pharmaceutical substance to a given depth below the surface of the skin

and consequently, clinical results can be variable. Thus, mechanical breach of the stratum corneum such as with needles, is believed to provide the most reproducible method of administration of substances through the surface of the skin, and to provide control and reliability in placement of administered substances.

[0004] Approaches for delivering substances beneath the surface of the skin have almost exclusively involved transdermal administration, *i.e.* delivery of substances through the skin to a site beneath the skin. Transdermal delivery includes subcutaneous, intramuscular or intravenous routes of administration of which, intramuscular (IM) and subcutaneous (SC) injections have been the most commonly used

[0005] Anatomically, the outer surface of the body is made up of two major tissue layers, an outer epidermis and an underlying dermis, which together constitute the skin (for review, see *Physiology, Biochemistry, and Molecular Biology of the Skin, Second Edition*, L.A. Goldsmith, Ed., Oxford University Press, New York, 1991). The epidermis is subdivided into five layers or strata of a total thickness of between 75 and 150 μm . Beneath the epidermis lies the dermis, which contains two layers, an outermost portion referred to as the papillary dermis and a deeper layer referred to as the reticular dermis. The papillary dermis contains vast microcirculatory blood and lymphatic plexuses. In contrast, the reticular dermis is relatively acellular and avascular and made up of dense collagenous and elastic connective tissue. Beneath the epidermis and dermis is the subcutaneous tissue, also referred to as the hypodermis, which is composed of connective tissue and fatty tissue. Muscle tissue lies beneath the subcutaneous tissue.

[0006] As noted above, both the subcutaneous tissue and muscle tissue have been commonly used as sites for administration of pharmaceutical substances. The dermis, however, has rarely been targeted as a site for administration of substances, and this may be due, at least in part, to the difficulty of precise needle placement into the intradermal space. Furthermore, even though the dermis, in particular, the papillary dermis has been known to have a high degree of vascularity, it has not heretofore been appreciated that one could take advantage of this high degree of vascularity to obtain an improved absorption profile for administered substances compared to subcutaneous administration. This is because small drug molecules are typically rapidly absorbed after administration into the subcutaneous tissue that has

been far more easily and predictably targeted than the dermis has been. On the other hand, large molecules such as proteins are typically not well absorbed through the capillary epithelium regardless of the degree of vascularity so that one would not have expected to achieve a significant absorption advantage over subcutaneous administration by the more difficult to achieve intradermal administration even for large molecules.

[0007] One approach to administration beneath the surface to the skin and into the region of the intradermal space has been routinely used in the Mantoux tuberculin test. In this procedure, a purified protein derivative is injected at a shallow angle to the skin surface using a 27 or 30 gauge needle and standard syringe (Flynn *et al.*, *Chest* 106: 1463-5, 1994). The Mantoux technique involves inserting the needle into the skin laterally, then “snaking” the needle further into the ID tissue. The technique is known to be quite difficult to perform and requires specialized training. A degree of imprecision in placement of the injection results in a significant number of false negative test results. Moreover, the test involves a localized injection to elicit a response at the site of injection and the Mantoux approach has not led to the use of intradermal injection for systemic administration of substances. Another group reported on what was described as an intradermal drug delivery device (U.S. Patent No. 5,997,501). Injection was indicated to be at a slow rate and the injection site was intended to be in some region below the epidermis, *i.e.*, the interface between the epidermis and the dermis or the interior of the dermis or subcutaneous tissue. This reference, however, provided no teachings that would suggest a selective administration into the dermis nor did the reference suggest that vaccines or gene therapeutic agents might be delivered in this manner. To date, numerous therapeutic proteins and small molecular weight compounds have been delivered intradermally and used to effectively elicit a pharmacologically beneficial response. Most of these compounds (*e.g.*, insulin, Neupogen, hGH, calcitonin) have been hormonal proteins not engineered receptors or antibodies. To date all administered proteins have exhibited several effects associated with ID administration, including more rapid onset of uptake and distribution (vs. SC) and in some case increased bioavailability.

[0008] Dermal tissue represents an attractive target site for delivery of vaccines and gene therapeutic agents. In the case of vaccines (both genetic and conventional), the skin is an attractive delivery site due to the high concentration of

antigen presenting cells (APC) and APC precursors found within this tissue, in particular the epidermal Langerhan's cells and dermal dendritic cells. Several gene therapeutic agents are designed for the treatment of skin disorders, skin diseases and skin cancer. In such cases, direct delivery of the therapeutic agent to the affected skin tissue is desirable. In addition, skin cells are an attractive target for gene therapeutic agents, of which the encoded protein or proteins are active at sites distant from the skin. In such cases, skin cells (*e.g.*, keratinocytes) can function as "bioreactors" producing a therapeutic protein that can be rapidly absorbed into the systemic circulation via the papillary dermis. In other cases, direct access of the vaccine or therapeutic agent to the systemic circulation is desirable for the treatment of disorders distant from the skin. In such cases, systemic distribution can be accomplished through the papillary dermis.

[0009] However, as discussed above, intradermal (ID) injection using standard needles and syringes is technically very difficult to perform and is painful. The prior art contains several references to ID delivery of both DNA-based and conventional vaccines and therapeutic agents, however results have been conflicting, at least in part due to difficulties in accurately targeting the ID tissue with existing techniques.

[0010] Virtually all of the human vaccines currently on the market are administered via the IM or SC routes. Of the 32 vaccines marketed by the 4 major global vaccine producers in the year 2001 (Aventis-Pasteur, GlaxoSmithKline, Merck, Wyeth), only 2 are approved for ID use (*2001 Physicians Desk Reference*). In fact, the product inserts for 6 of these 32 vaccines specifically states *not* to use the ID route. This is despite the various published pre-clinical and early clinical studies suggesting that ID delivery can improve vaccines by inducing a stronger immune response than via IM or SC injection or by inducing a comparable immune response at a reduced dose relative to that which is given IM or SC (Playford, *E. G. et al.*, 2002, *Infect. Control Hosp. Epidemiol.* 23:87; Kerr, C. 2001, *Trends Microbiol.* 9:415; Rahman, F. *et al.*, 2000, *Hepatology* 31:521; Carlsson, U. *et al.*, 1996, *Scan J. Infect. Dis.* 28:435; Propst, T. *et al.*, 1998, *Amer. J. Kidney Dis.* 32:1041; Nagafuchi, S. *et al.*, 1998, *Rev Med Virol.*, 8:97; Henderson, E.A., *et al.*, 2000. *Infect. Control Hosp Epidemiol.* 21:264). Although improvements in vaccine efficacy following ID delivery have been noted in some cases, others have failed to observe such advantages

(Crowe, 1965, *Am. J. Med. Tech.* 31:387-396; Letter to British Medical Journal 29/10/77, p. 1152; Brown *et al.*, 1977, *J. Infect. Dis.* 136:466-471; Herbert & Larke, 1979, *J. Infect. Dis.* 140:234-238; Ropac *et al. Periodicum Biologorum* 2001, 103:39-43).

[0011] A major factor that has precluded the widespread use of the ID delivery route and has contributed to the conflicting results described above is the lack of suitable devices to accomplish reproducible delivery to the epidermal and dermal skin layers. Standard needles commonly used to inject vaccines are too large to accurately target these tissue layers when inserted into the skin. The most common method of delivery is through Mantoux-style injection using a standard needle and syringe. This technique is difficult to perform, unreliable and painful to the subject. Thus, there is a need for devices and methods that will enable efficient, accurate and reproducible delivery of vaccines and gene therapeutic agents to the intradermal layer of skin.

2. SUMMARY OF THE INVENTION

[0012] The present invention improves the clinical utility of ID delivery of vaccines and gene therapeutic agents to humans or animals. The methods employ devices to directly target the intradermal space and to deliver substances to the intradermal space as a bolus or by infusion. It has been discovered that the placement of the substance within the dermis provides for efficacious and/or improved responsiveness to vaccines and gene therapeutic agents. The device is so designed as to prevent leakage of the substance from the skin and improve adsorption or cellular uptake within the intradermal space. The immunological response to a vaccine delivered according to the methods of the invention has been found to be equivalent to or improved over conventional IM delivery of the vaccine, indicating that ID administration according to the methods of the invention will in many cases provide improved clinical results, in addition to the other advantages of ID delivery.

[0013] The present disclosure also relates to methods and devices for delivering vaccines or genetic material to an individual based on directly targeting the dermal space whereby such method allows improved delivery and/or an improved response to the vaccine or genetic material. By the use of direct intradermal (ID) administration means (hereafter referred to as dermal-access means), for example

using microneedle-based injection and infusion systems, or other means to accurately target the intradermal space, the efficacy of many substances including vaccines and gene therapy agents can be improved when compared to traditional parental administration routes of subcutaneous and intramuscular delivery.

[0014] Accordingly, it is one object of the invention to provide a method to accurately target the ID tissue to deliver a vaccine or a medicament comprising genetic material to afford an immunogenic or therapeutic response.

[0015] It is a further object of the invention to provide a method to improve the systemic immunogenic or therapeutic response to vaccine (conventional or genetic) or medicament comprising genetic material by accurately targeting the ID tissue

[0016] Yet another object of the invention is to provide a method to improve the availability of a vaccine (conventional or genetic) to APC residing in the skin in order to effectuate an antigen-specific immune response to the vaccine by accurately targeting the ID tissue. This may, in many cases, allow for smaller doses of the substance to be administered via the ID route.

[0017] Yet another object of the present invention is to provide a method to improve the delivery of a medicament comprising genetic material for the treatment of skin diseases, genetic skin disorders or skin cancer by accurately targeting the ID tissue. The resultant genetic material is subsequently expressed by the cells within the targeted ID tissue.

[0018] Yet another object of the present invention is to provide a method to improve the delivery of a medicament comprising genetic material for the treatment of diseases, genetic disorders, or cancers affecting tissues distant from the skin by accurately targeting the ID tissue. The resultant genetic material is subsequently expressed by the cells within the targeted ID tissue, distant therefrom or both.

[0019] These and other benefits of the invention are achieved by directly targeting delivery of the substance to the preferred depth for the particular therapeutic or prophylactic agent. The inventors have found that by specifically targeting delivery of the substance to the intradermal space, the response to vaccines and gene therapeutic agents can be unexpectedly improved, and can in many situations be varied with resulting clinical advantage.

3. BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1 shows reporter gene activity in guinea pig skin following delivery of plasmid DNA encoding firefly luciferase. Results are shown as relative light units (RLU) per mg protein for intradermal delivery by the Mantoux method, the delivery method of the invention, and control group in which topical application of the Plasmid DNA was made to shaved skin.

[0021] Figure 2 shows reporter gene activity in rat skin following delivery of plasmid DNA encoding firefly luciferase. Results are shown as RLU/mg protein for intradermal delivery by the microdermal delivery method (one embodiment of the invention, MDD), and control group in which an unrelated plasmid DNA was injected.

[0022] Figure 3 shows reporter gene activity in pig skin following delivery of plasmid DNA encoding β -galactosidase. Results are shown as RLU/mg protein for intradermal delivery by the Mantoux method, by ID delivery via perpendicular insertion into skin using MDD device (34g) or 30g needle to depths of 1 mm and 1.5 mm, respectively, and negative control.

[0023] Figure 4 shows total protein content at recovered skin sites in pigs following Mantoux ID and MDD delivery of reporter plasmid DNA. Control ("Negative") is untreated skin.

[0024] Figure 5 shows the influenza-specific serum antibody response in rats following delivery of plasmid DNA encoding influenza virus hemagglutinin in the absence of added adjuvant. Plasmid DNA was administered via ID delivery with the MDD device or via intra-muscular (IM) injection with a standard needle and syringe. "Topical" indicates control group, where the preparation was topically applied to skin.

[0025] Figure 6 shows the influenza-specific serum antibody response in rats following delivery of plasmid DNA encoding influenza virus hemagglutinin in the presence of adjuvant. Plasmid DNA was administered via ID delivery with the

MDD device or via intra-muscular (IM) injection with a standard needle and syringe. "Topical" indicates control group, where the preparation was topically applied to skin.

[0026] Figure 7 shows the influenza-specific serum antibody response in rats following “priming” with plasmid DNA in the absence of added adjuvant followed by “boosting” with whole inactivated influenza virus in the absence of added adjuvant. Plasmid DNA or whole inactivated influenza virus was administered via ID delivery with the MDD device or via intra-muscular (IM) injection with a standard needle and syringe. "Topical" indicates control group, where the preparation was topically applied to skin.

[0027] Figure 8 shows the influenza-specific serum antibody response in rats following “priming” with plasmid DNA in the presence of added adjuvant followed by “boosting” with whole inactivated influenza virus in the absence of added adjuvant. Plasmid DNA or whole inactivated influenza virus was administered via ID delivery with the MDD device or via intra-muscular (IM) injection with a standard needle and syringe. "Topical" indicates control group, where the preparation was topically applied to skin.

[0028] Figure 9 shows the influenza-specific serum antibody response in rats to a whole inactivated influenza virus preparation administered via ID delivery with the MDD device or via intra-muscular (IM) injection with a standard needle and syringe. "Topical" indicates control group, where the preparation was topically applied to skin.

[0029] Figure 10 shows the influenza-specific serum antibody response in pigs to a whole inactivated influenza virus preparation administered via ID delivery with the MDD device or via intra-muscular (IM) injection with a standard needle and syringe.

[0030] Figure 11 shows the influenza-specific serum antibody response in rats to reduced doses of a whole inactivated influenza virus preparation

administered via ID delivery with the MDD device or via IM injection with a standard needle and syringe.

4. DETAILED DESCRIPTION OF THE INVENTION

[0031] The present invention improves the clinical utility of ID delivery of vaccines and gene therapeutic agents to humans or animals. The methods encompass devices to directly target the intradermal space and to deliver substances to the intradermal space as a bolus or by infusion. It has been discovered that the placement of the substance within the dermis provides for efficacious and/or improved responsiveness to vaccines and gene therapeutic agents. The device is so designed as to prevent leakage of the substance from the skin and improve adsorption or cellular uptake within the intradermal space. The immunological response to a vaccine delivered according to the methods of the invention has been found to be equivalent to or improved over conventional IM delivery of the vaccine, indicating that ID administration according to the methods of the invention will in many cases provide improved clinical results, in addition to the other advantages of ID delivery.

[0032] As used herein, "intradermal" (ID) is intended to mean administration of a substance into the dermis in such a manner that the substance readily reaches the richly vascularized papillary dermis where it can be rapidly systemically absorbed, or in the case of vaccines (conventional and genetic) or gene therapeutic agents may be taken up directly by cells in the skin. In the case of genetic vaccines, intended target cells include APC (including epidermal Langerhan's cells and dermal dendritic cells). In the case of gene therapeutic agents for diseases, genetic disorders or cancers affecting tissues distant from the skin, intended target cells include keratinocytes or other skin cells capable of expressing a therapeutic protein. In the case of gene therapeutic agents for diseases, genetic disorders or cancers affecting the skin, the intended target cells include those skin cells which may be affected by the disease, genetic disorder or cancer.

[0033] As used herein, "targeted delivery" means delivery of the substance to the target depth, and includes delivery that may result in the same response in a treated individual, but result in less pain, more reproducibility, or other

advantage compared to an alternate accepted means of delivery (*e.g.*, topical, subcutaneous or intramuscular).

[0034] As used herein, an “improved response” includes an equivalent response to a reduced amount of compound administered or an increased response to an identical amount of compound that is administered by an alternate means of delivery or any other therapeutic or immunological benefit.

[0035] The terms "needle" and "needles" as used herein are intended to encompass all such needle-like structures. The terms microcannula or microneedles, as used herein, are intended to encompass structures smaller than about 31 gauge, typically about 31-50 gauge when such structures are cylindrical in nature. Non-cylindrical structures encompassed by the term microneedles would be of comparable diameter and include pyramidal, rectangular, octagonal, wedged, and other geometrical shapes.

[0036] As used herein, the term “bolus” is intended to mean an amount that is delivered within a time period of less than ten (10) minutes. A "rapid bolus" is intended to mean an amount that is delivered in less than one minute. “Infusion” is intended to mean the delivery of a substance over a time period greater than ten (10) minutes.

[0037] The term "nucleic acids" includes polynucleotides, RNA, DNA, or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form, and may be of any size that can be formulated and delivered using the methods of the present invention. Nucleic acids may be of the “antisense” type. By "nucleic acid derived entity" is meant an entity composed of nucleic acids in whole or in part.

[0038] By "gene therapeutic agent" is meant an agent that is intended to be delivered into or be capable of uptake by cell(s) of the treated individual for incorporation and expression of genetic material. The gene therapeutic agent will ordinarily include a polynucleotide that encodes a peptide, polypeptide, protein or

glycoprotein of interest, optionally contained in a vector or plasmid, operationally linked to any further nucleic acid sequences necessary for expression.

[0039] When referring to the administration of vaccines or gene therapeutic agents, the term "simultaneously" is generally means the administration of two dosages within the same 24 hour period, whereas "sequentially" or "subsequently" is intended to mean that the dosages are separated by more than 24 hours. It will be appreciated by those of skill in the art that simultaneous administration will generally refer to dosages administered at the same medical visit, whereas subsequently or sequentially will refer to dosages that may be separated by days, weeks, months, and occasionally years, depending on the effects of a particular vaccine or gene therapeutic. In one preferred embodiment, "sequential" or "subsequent" refers to dosages that are separated by one day to six weeks.

[0040] The desired therapeutic or immunogenic response is directly related to the ID targeting depth. These results can be obtained by placement of the substance in the upper region of the dermis, *i.e.*, the papillary dermis or in the upper portion of the relatively less vascular reticular dermis such that the substance readily diffuses into the papillary dermis. Placement of a substance predominately at a depth of at least about 0.025 mm to about 2.5 mm is preferred.

4.1 DELIVERY AND ADMINISTRATION OF VACCINES AND GENE THERAPEUTIC AGENTS

[0041] The invention encompasses delivering a vaccine or gene therapeutic agents to the intradermal space of a subject's skin. In particular, for vaccines, it is preferred that delivery be at a targeted depth of just under the stratum corneum and encompassing the epidermis and upper dermis (about 0.025 mm to about 2.5 mm). For therapeutics that target cells in the skin, the preferred target depth depends on the particular cell being targeted; for example to target the Langerhans cells, delivery would need to encompass at least in part the epidermal tissue depth typically ranging from about 0.025 mm to about 0.2 mm in humans. For therapeutics and vaccines that require systemic circulation, the preferred target depth would be between, at least about 0.4 mm and most preferably at least about 0.5 mm up to a

depth of no more than about 2.5 mm, more preferably, no more than about 2.0 mm and most preferably no more than about 1.7 mm will result delivery of the substance to the desired dermal layer. Placement of the substance predominately at greater depths and/or into the lower portion of the reticular dermis is usually considered to be less desirable.

[0042] The dermal-access means used for ID administration according to the invention is not critical as long as it provides the insertion depth into the skin of a subject necessary to provide the targeted delivery depth of the substance. In most cases, the device will penetrate the skin and to a depth of about 0.5-2 mm. The dermal-access means may comprise conventional injection needles, catheters, microcannula or microneedles of all known types, employed singularly or in multiple needle arrays.

[0043] By varying the targeted depth of delivery of substances by the dermal-access means, behavior of the drug or substance can be tailored to the desired clinical application most appropriate for a particular patient's condition. The targeted depth of delivery of substances by the dermal-access means may be controlled manually by the practitioner, or with or without the assistance of indicator means to indicate when the desired depth is reached. Preferably however, the device has structural means for controlling skin penetration to the desired depth within the intradermal space. This is most typically accomplished by means of a widened area or hub associated with the dermal-access means that may take the form of a backing structure or platform to which the needles are attached. The length of microneedles as dermal-access means are easily varied during the fabrication process and are routinely produced. Microneedles are also very sharp and of a very small gauge, to further reduce pain and other sensation during the injection or infusion. They may be used in the invention as individual single-lumen microneedles or multiple microneedles may be assembled or fabricated in linear arrays or two-dimensional arrays as to increase the rate of delivery or the amount of substance delivered in a given period of time. Microneedles having one or more sideports are also included as dermal access means. Microneedles may be incorporated into a variety of devices such as holders and housings that may also serve to limit the depth of penetration. The dermal-access

means of the invention may also incorporate reservoirs to contain the substance prior to delivery or pumps or other means for delivering the drug or other substance under pressure. Alternatively, the device housing the dermal-access means may be linked externally to such additional components. The dermal-access means may also include safety features, either passive or active, to prevent or reduce accidental injury.

[0044] In one embodiment of the invention, ID injection can be reproducibly accomplished using one or more narrow gauge microcannula inserted perpendicular to the skin surface. This method of delivery ("microdermal delivery" or "MDD") is easier to accomplish than standard Mantoux-style injections and, by virtue of its limited and controlled depth of penetration into the skin, is less invasive and painful. Furthermore, similar or greater biological responses, as measured here by gene expression and immune response, can be attained using the MDD devices compared to standard needles. Optimal depth for administration of a given substance in a given species can be determined by those of skill in the art without undue experimentation.

[0045] Delivery devices that place the dermal-access means at an appropriate depth in the intradermal space, control the volume and rate of fluid delivery and provide accurate delivery of the substance to the desired location without leakage are most preferred. Micro-cannula- and microneedle-based methodology and devices are described in EP 1 092 444 A1, and U.S. Application Serial No. 606,909, filed June 29, 2000. Standard steel cannula can also be used for intra-dermal delivery using devices and methods as described in U.S. Serial No. 417,671, filed October 14, 1999, the contents of each of which are expressly incorporated herein by reference. These methods and devices include the delivery of substances through narrow gauge (about 30G) "micro-cannula" with limited depth of penetration, as defined by the total length of the cannula or the total length of the cannula that is exposed beyond a depth-limiting feature. These methods and devices provide for the delivery of substances through 30 or 31 gauge cannula, however, the present invention also employs 34G or narrower "microcannula" including if desired, limited or controlled depth of penetration means. It is within the scope of the present invention that targeted delivery of substances can be achieved either through a single microcannula or an

array of microcannula (or "microneedles"), for example 3-6 microneedles mounted on an injection device that may include or be attached to a reservoir in which the substance to be administered is contained.

[0046] Using the methods of the present invention, vaccines and gene therapeutic agents may be administered as a bolus, or by infusion. It is understood that bolus administration or delivery can be carried out with rate controlling means, for example a pump, or have no specific rate controlling means, for example, user self-injection. The above-mentioned benefits are best realized by accurate direct targeted delivery of substances to the dermal tissue compartment including the epidermal tissue. This is accomplished, for example, by using microneedle systems of less than about 250 micron outer diameter, and less than 2 mm exposed length. By "exposed length" it is meant the length of the narrow hollow cannula or needle available to penetrate the skin of the patient. Such systems can be constructed using known methods for various materials including steel, silicon, ceramic, and other metals, plastic, polymers, sugars, biological and or biodegradable materials, and/or combinations thereof.

[0047] It has been found that certain features of the intradermal administration methods provide the most efficacious results. For example, it has been found that placement of the needle outlet within the skin significantly affects the clinical response to delivery of a vaccine or gene therapy agent. The outlet of a conventional or standard gauge needle with a bevel angle cut to 15 degrees or less has a relatively large "exposed height". As used herein the term exposed height refers to the length of the opening relative to the axis of the cannula resulting from the bevel cut. When standard needles are placed at the desired depth within the intradermal space (at about 90 degrees to the skin), the large exposed height of these needle outlets causes the substance usually to effuse out of the skin due to backpressure exerted by the skin itself and to pressure built up from accumulating fluid from the injection or infusion. Typically, the exposed height of the needle outlet of the present invention is from 0 to about 1 mm. A needle outlet with an exposed height of 0 mm has no bevel cut (or a bevel angle of 90 degrees) and is at the tip of the needle. In this case, the depth of the outlet is the same as the depth of penetration of the needle. A

needle outlet that is either formed by a bevel cut or by an opening through the side of the needle has a measurable exposed height. In a needle having a bevel, the exposed height of the needle outlet is determined by the diameter of the needle and the angle of the primary bevel cut ("bevel angle"). In general, bevel angles of greater than 20° are preferred, more preferably between 25° and 40°. It is understood that a single needle may have more than one opening or outlet suitable for delivery of substances to the dermal space.

[0048] Thus the exposed height, and for the case of a cannula with an opening through the side, its position along the axis of the cannula contributes to the depth and specificity at which a substance is delivered. Additional factors taken alone or in combination with the cannula, such as delivery rate and total fluid volume delivered, contribute to the target delivery of substances and variation of such parameters to optimize results is within the scope of the present invention.

[0049] It has also been found that controlling the pressure of injection or infusion may avoid the high backpressure exerted during ID administration. By placing a constant pressure directly on the liquid interface a more constant delivery rate can be achieved, which may optimize absorption and obtain an improved response for the dosage of vaccine or therapeutic agent delivered. Delivery rate and volume can also be controlled to prevent the formation of wheals at the site of delivery and to prevent backpressure from pushing the dermal-access means out of the skin. The appropriate delivery rates and volumes to obtain these effects for a selected substance may be determined experimentally using only ordinary skill and without undue experimentation. Increased spacing between multiple needles allows broader fluid distribution and increased rates of delivery or larger fluid volumes.

[0050] In one embodiment, to deliver a substance the dermal-access means is placed adjacent to the skin of a subject providing directly targeted access within the intradermal space and the substance or substances are delivered or administered into the intradermal space where they can act locally or be absorbed by the bloodstream and be distributed systemically. In another embodiment, the dermal-access means is positioned substantially perpendicular to the skin surface to provide

vertical insertion of one or more cannula. The dermal-access means may be connected to a reservoir containing the substance or substances to be delivered. The form of the substance or substances to be delivered or administered include solutions thereof in pharmaceutically acceptable diluents or solvents, emulsions, suspensions, gels, particulates such as micro- and nanoparticles either suspended or dispersed, as well as in-situ forming vehicles of the same. Delivery from the reservoir into the intradermal space may occur either passively, without application of the external pressure or other driving means to the substance or substances to be delivered, and/or actively, with the application of pressure or other driving means. Examples of preferred pressure generating means include pumps, syringes, elastomer membranes, gas pressure, piezoelectric, electromotive, electromagnetic pumping, coil springs, or Belleville springs or washers or combinations thereof. If desired, the rate of delivery of the substance may be variably controlled by the pressure-generating means. As a result, the substance enters the intradermal space and is absorbed in an amount and at a rate sufficient to produce a clinically efficacious result.

4.2 VACCINES AND GENE THERAPEUTIC AGENTS

[0051] Substances that may be delivered according to the methods of the invention include vaccines, with or without carriers, adjuvants and vehicles, including prophylactic and therapeutic antigens including but not limited to subunit proteins, peptides and polysaccharides, polysaccharide conjugates, toxoids, genetic based vaccines, live attenuated bacteria or viruses, mutated bacteria or viruses, reassortant bacteria or viruses, inactivated bacteria or viruses, whole cells or components thereof (*e.g.*, mammalian cells), cellular vaccines (*e.g.*, autologous dendritic cells), or components thereof (for example, exosomes, dexosomes, membrane fragments, or vesicles), live viruses, live bacteria, viral and bacterial vectors including but not limited to those derived from adenoviruses, retroviruses alphaviruses, flaviviruses, and vaccinia viruses) in connection with addiction (*e.g.*, cocaine addiction), anthrax, arthritis, cholera, diphtheria, dengue, tetanus, lupus, multiple sclerosis, parasitic diseases, psoriasis, Lyme disease, meningococcus, measles, mumps, rubella, varicella, yellow fever, Respiratory syncytial virus, tick borne Japanese encephalitis, pneumococcus, smallpox, streptococcus, staphylococcus, typhoid, influenza, hepatitis, including hepatitis A, B, C and E, otitis media, rabies,

polio, HIV, parainfluenza, rotavirus, Epstein Barr Virus, CMV, chlamydia, non-typeable haemophilus, haemophilus influenza B (HIB), moraxella catarrhalis, human papilloma virus, tuberculosis including BCG, gonorrhoeae, asthma, atherosclerosis, malaria, *E. coli*, Alzheimer's Disease, *H. Pylori*, salmonella, diabetes, cancer, herpes simplex, human papilloma, *Yersinia pestis*, traveler's diseases, West Nile encephalitis, *Camplobacter*, *C. difficile*.

[0052] The vaccines used in the methods and compositions of the invention comprise one or more antigenic or immunogenic agent, against which an immune response is desired. In certain embodiments, the vaccine formulations of the invention comprise recombinant or chimeric viruses encoded by viral vectors derived from the genome of a virus, such as those listed *supra*. In accordance with the invention a recombinant virus is encoded by endogenous or native genomic sequences and/or non-native genomic sequences of a virus. A native or genomic sequence is one that is different from the native or endogenous genomic sequence due to one or more mutations, including, but not limited to, point mutations, rearrangements, insertions, deletions *etc.*, to the genomic sequence that may or may not result in a phenotypic change. The recombinant viruses of the invention encompass those viruses encoded by viral vectors derived from the genomes of a virus, such as those listed *supra*, and may or may not, include nucleic acids that are non-native to the viral genome. In accordance with the invention, a chimeric virus may be encoded by a nucleotide sequence in which heterologous nucleotide sequences have been added to the genome or in which endogenous or native nucleotide sequences have been replaced with heterologous nucleotide sequences.

[0053] In certain embodiments, completely foreign epitopes, including antigens derived from other viral or non-viral pathogens can be engineered into the virus for use in the vaccine formulations of the invention. For example, antigens of viruses such as HIV (*e.g.*, gp160, gp120, gp41, influenza, hepatitis, including hepatitis A, B, C and E, rabies, polio, HIV, parainfluenza, rotavirus, Epstein Barr Virus, CMV, flaviviruses including but not limited to, Japanese Encephalitis (JE, *e.g.*, JE SA14-14-2), Dengue (DEN, *e.g.*, any of the Dengue serotypes 1-4); Murray Valley encephalitis, St Louis Encephalitis, West Nile, Tick borne encephalitis, Hepatitis C viruses, Kunjin virus, Powassan virus, Kyasanur Forest Disease virus, and Omsk Hemorrhagic Fever

Virus, parasite antigens (e.g., malaria), bacterial or fungal antigens or tumor antigens can be engineered into the attenuated strain.

[0054] Virtually any heterologous gene sequence may be constructed into the chimeric viruses of the invention for use in the vaccine formulations. Preferably, heterologous gene sequences are moieties and peptides that act as biological response modifiers. Preferably, epitopes that induce a protective immune response to any of a variety of pathogens, or antigens that bind neutralizing antibodies may be expressed by or as part of the chimeric viruses. For example, heterologous gene sequences that can be constructed into the chimeric viruses of the invention include, but are not limited to, influenza and parainfluenza hemagglutinin neuraminidase and fusion glycoproteins such as the HN and F genes of human PIV3. In yet another embodiment, heterologous gene sequences that can be engineered into the chimeric viruses include those that encode proteins with immuno-modulating activities. Examples of immuno-modulating proteins include, but are not limited to, cytokines, interferon type 1, gamma interferon, colony stimulating factors, interleukin -1, -2, -4, -5, -6, -12, and antagonists of these agents.

[0055] Other heterologous sequences may be derived from tumor antigens, and the resulting chimeric viruses be used to generate an immune response against the tumor cells leading to tumor regression *in vivo*. In accordance with the present invention, recombinant viruses may be engineered to express tumor-associated antigens (TAAs), including but not limited to, human tumor antigens recognized by T cells (Robbins and Kawakami, 1996, *Curr. Opin. Immunol.* 8:628-636, incorporated herein by reference in its entirety), melanocyte lineage proteins, including gp100, MART-1/MelanA, TRP-1 (gp75), tyrosinase; Tumor-specific widely shared antigens, MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-1, N-acetylglucosaminyltransferase-V, p15; Tumor-specific mutated antigens, β -catenin, MUM-1, CDK4; Nonmelanoma antigens for breast, ovarian, cervical and pancreatic carcinoma, HER-2/neu, human papillomavirus -E6, -E7, MUC-1.

[0056] In a preferred specific embodiment, the chimeric vaccines of the invention comprise recombinant chimeric flaviviruses encoded by viral vectors derived from genomes of yellow fever virus and one or more other flaviviruses. In a specific embodiment, the chimeric flaviviruses for use in the methods and compositions of the invention are preferably live, infectious, attenuated virus,

comprising: (1) a first flavivirus, preferably a yellow fever virus, *e.g.*, strain 17D, which is preferably a live attenuated vaccine virus, in which the nucleotide sequence encoding an envelope protein, *e.g.*, the prM and E protein, is modified, for example by deletion, truncation or mutation so that the functional envelope protein of the first flavivirus is not expressed; (2) a nucleotide sequence encoding the viral envelope protein of a second flavivirus, *i.e.*, Dengue virus, which is different from the first flavivirus, so that the envelope protein of the second flavivirus is expressed. The invention encompasses chimeric flavivirus vaccines known in the art, such as those disclosed in International Publication No. WO 01/39802, which is incorporated herein by reference in its entirety. In a preferred embodiment, the prM and E protein nucleotide encoding sequence of YF is replaced with the prM and E protein nucleotide encoding sequence of a second flavivirus.

[0057] A preferred live virus for use as the first yellow fever in the chimeric vaccines of the invention is YF 17D, see, *e.g.*, Smithburn *et al.*, *Yellow Fever Vaccination, World Health Org.* p. 238, 1956; Freestone, in Plotkin *et al.*, eds, *Vaccines*, 2nd ed, W.B, Saunders, PA, 1995). YF has been studied at the genetic level (Rice *et al.* 1985, *Science* 229, 726-33). Other YF strains that may be used in the chimeric vaccine formulations of the invention include but are not limited to, YF 17DD (Genbank Accession No. U17066); YF-17D213 (Genbank Accession No. U17067), YF 17D-204 France (Genbank Accession No. X15067, X15062), and YF 17D-204, 234 US (Rice *et al.*, 1985, *Science* 229, 726-33; Rice *et al.*, *New Biologist*, 1: 285-96; Genbank Accession No. C03700; K 02749). Other Yellow fever strains encompassed within the invention are described by Gallery *et al.*, 1998, *Vaccine*, 16: 1024-28, which is incorporated herein by reference in its entirety.

[0058] Preferred flaviviruses for use as the second flavivirus in the chimeric viruses of the invention, include but are not limited to Japanese Encephalitis (JE, *e.g.*, JE SA14-14-2), Dengue (DEN, *e.g.*, any of the Dengue serotypes 1-4); Murray Valley encephalitis (MVE), St Louis Encephalitis (SLE), West Nile (WN), Tick borne encephalitis (TBE), Hepatitis C viruses, Kunjin virus, Powassan virus, Kyasanur Forest Disease virus, and Omsk Hemorrhagic Fever Virus.

[0059] In some embodiments, the second flavivirus envelope protein nucleotide encoding sequence in the chimeric flavivirus vaccines is derived from two different second flaviviruses, *e.g.*, two Dengue strains. In some embodiments, the

second flavivirus envelope protein nucleotide encoding sequence in the chimeric flavivirus vaccines is attenuated using methods known to one skilled in the art. When the second flavivirus is a neurotropic virus, such as Japanese Encephalitis, Murray Valley encephalitis (MVE), St Louis Encephalitis (SLE), the envelope protein nucleotide encoding sequence is preferably attenuated. In the case of non-neurotropic viruses, *e.g.*, dengue virus, it may be preferable to use envelope protein nucleotide encoding sequences that are not attenuated. Although not intending to be bound by a particular mechanism of action, maintenance of native sequences may lead to enhanced immunogenicity, and thus a more effective vaccine.

[0060] In a preferred embodiment, the second flavivirus envelope protein nucleotide encoding sequence in the chimeric flavivirus vaccines is derived from two different second flaviviruses, *e.g.*, two different Dengue strains. In some embodiments, the second flavivirus envelope protein nucleotide encoding sequence in the chimeric flavivirus vaccines is attenuated using methods known to one skilled in the art. In the case of non-neurotropic viruses, *e.g.* dengue virus, it may be preferable to use envelope protein nucleotide encoding sequences that are not attenuated. Although not intending to be bound by a particular mechanism of action, maintenance of native sequences may lead to enhanced immunogenicity, and thus a more effective vaccine.

[0061] The chimeric flavivirus vaccines of the invention are constructed using common recombinant DNA methodologies known to one skilled in the art. Preferably, the chimeric flavivirus vaccines of the invention are constructed in accordance with methods described in International Publication No. WO 01/39802, which is incorporated herein by reference in its entirety. In particular, the chimeric flavivirus vaccines of the invention are constructed using the principles set forth in International Publication No. WO 01/39802, ensuring proper proteolytic processing of the polypeptide. YF viral proteins are produced by translation of a single long open reading frame to generate a polyprotein. A complex series of post-translational proteolytic processing coupled with host and viral proteases generate the mature viral proteins. The structural proteins are arranged in the order C-prM-E, where C is cased, prM is a precursor of the viral envelope bound M protein, and E is the envelope protein. A stretch of about 20 amino acids separates C-prM and is referred to as the prM signal sequence which is bound by the two proteolytic sites resulting in the

release of the C and prM proteins. The amino terminus of prM is generated by host signalase cleavage within the lumen of the ER, and the NS2B-NS3 protease complex is responsible for mediating cleavage at the C terminus of the C protein. Maintenance of a coordinated cleavage of NS2B-NS3 protease complex at the C-terminus of the C protein and the signalase at the N terminus of the prM protein is critical for proper processing of the polyprotein. In particular, in the chimeric vaccines of the invention the length of the prM signal sequence separating the two cleavage sites is substantially maintained to ensure proper processing and subsequent viability of the chimeric viruses. In most preferred embodiments, in constructing the chimeric flavivirus of the invention the prM signal of the YF backbone is maintained. Preferably, when a sequence from the second flavivirus is introduced into the YF backbone, it is inserted after prM signal sequence. Preferably, the length and sequence of the YF prM signal is maintained. In some embodiments, the YF prM signal sequence may be modified by conservative amino acid substitutions.

[0062] The invention encompasses vaccine formulations in which the virus is attenuated. Production of recombinant, chimeric and attenuated viruses may be performed using standard methods known to one skilled in the art. The invention encompasses a live recombinant viral vaccine or an inactivated recombinant viral vaccine to be formulated in accordance with the invention. A live vaccine may be preferred because multiplication in the host leads to a prolonged stimulus of similar kind and magnitude to that occurring in natural infections, and therefore, confers substantial, long-lasting immunity. Production of such live recombinant virus vaccine formulations may be accomplished using conventional methods involving propagation of the virus in cell culture or in the allantois of the chick embryo followed by purification.

[0063] In a specific embodiment, the recombinant virus used in the vaccine formulations of the invention is non-pathogenic to the subject to which it is administered. In this regard, the use of genetically engineered viruses for vaccine purposes may require the presence of attenuation characteristics in these strains. The introduction of appropriate mutations (*e.g.*, deletions) into the templates used for transfection may provide the novel viruses with attenuation characteristics. For example, specific missense mutations which are associated with temperature sensitivity or cold adaption can be made into deletion mutations. These mutations

should be more stable than the point mutations associated with cold or temperature sensitive mutants and reversion frequencies should be extremely low.

[0064] Alternatively, chimeric viruses with “suicide” characteristics may be constructed for use in the vaccine formulations of the invention. Such viruses would go through only one or a few rounds of replication within the host. When used as a vaccine, the recombinant virus would go through limited replication cycle(s) and induce a sufficient level of immune response but it would not go further in the human host and cause disease.

[0065] Alternatively, inactivated (killed) virus may be formulated in accordance with the invention. Inactivated vaccine formulations may be prepared using conventional techniques to “kill” the chimeric viruses. Inactivated vaccines are “dead” in the sense that their infectivity has been destroyed. Ideally, the infectivity of the virus is destroyed without affecting its immunogenicity. In order to prepare inactivated vaccines, the chimeric virus may be grown in cell culture or in the allantois of the chick embryo, purified by zonal ultracentrifugation, inactivated by formaldehyde or β -propiolactone, and pooled.

[0066] Suitable exemplary compositions for genetic immunization are described, for example, in U.S. Pat. Nos. 5,589,466, 5,593,972 and 5,703,055, all of which are incorporated herein by reference in their entireties.

[0067] Particularly preferred substances that can be delivered according to the methods of the invention include nucleic acids, nucleic acid derived entities and gene therapeutic agents and the like used in the prevention, diagnosis, alleviation, treatment, or cure of disease. Suitable adjuvants for inclusion in vaccines are known to those of skill in the art. Additional agents for enhancing immune response that may be used in the present invention are disclosed in U.S. application no. 10/142,966, filed May 13, 2002, which is incorporated herein by reference in its entirety.

[0068] Particularly preferred gene therapeutic agents include those indicated for the treatment of cancer including but not limited to melanoma, cutaneous T cell lymphoma, Kaposi’s sarcoma, cutaneous squamous cell carcinoma and basal cell carcinoma, adenosine deaminase deficiency, hyperproliferative skin diseases including but not limited to psoriasis, genetic skin diseases including but not limited to epidermolytic hyperkeratosis, epidermolysis bullosa, lamellar ichthyosis

and X-linked ichthyosis, hemophilia, cystic fibrosis, growth disorders, hormone deficiencies including but not limited to human growth hormone deficiency, atherosclerosis, transferrin deficiency, as well as gene therapeutic agents indicated for wound healing and tissue regeneration. Suitable exemplary compositions for suitable genetic therapeutic agents are described, for example, in U.S. Pat. No. 5,547,932, which is incorporated herein by reference in its entirety.

[0069] The substance may be delivered into the skin in any pharmaceutically acceptable form. Vaccines to be used in the methods of the invention may include adjuvants and carriers or vehicles that are suitable in particular formulations, as will be familiar to those of skill in the art.

[0070] Pharmaceutically acceptable peptide and polypeptide formulations for use in the invention, including formulations for allergen compositions, are also well known in the art. Nucleic acids for use in the methods of the invention may be RNA or DNA, or a combination thereof. They may be in any physical form suitable for ID administration and for uptake and expression by cells. DNA and/or RNA may be contained in a viral vector or liposome, or may be delivered as a free polynucleotide such as a plasmid as is known in the art. The nucleic acid will typically be formulated in a pharmaceutically acceptable formulation such as a fluid, gel, or suspension that is compatible with the nucleic acid.

5. KITS

[0071] Typically, to administer vaccine or other medicament a practitioner will remove the appropriate volume from a vial sealed with a septa using a syringe. This same syringe is then used administer the vaccine to the patient. However, a microneedle or microcannula, typically between 0.1 and 2 mm in length, in addition to being somewhat unsuitable in length to completely penetrate the septa, is generally too fragile to puncture a septum of a vial to extract medicament while maintaining sufficient sharpness and straightness to subsequently be used on a patient. Use of such microdevices in puncturing septa also may result in clogging of the bore of the needle. In addition, the narrow gauge, typically 31 to 50 gauge, of the microcannula greatly reduces the volumetric capacity that can traverse the needle into the syringe, for example. This would be inconvenient to most practitioners who are accustomed to rapid transfer of liquids from vials using conventional devices and thus would greatly increase the amount of time the practitioner would spend with the

patient. Additional factors to be considered in the widespread use of microdevices include the necessity to reformulate most drugs and vaccines to accommodate the reduced total volume (10-100 μ l) used or delivered by microdevices. Thus it would be desirable to provide for a kit including the device either in combination with or adapted to integrate therewith, the substance to be delivered.

[0072] Kits and the like comprising the instrument of administration and the therapeutic composition are well known in the art. However, the application of minimally invasive, ID microdevices for the delivery of drugs and vaccines clearly present an immediate need for coupling the device with the formulation to provide safe, efficacious, and consistent means for administering formulations for enabling immunogenic and therapeutic responses.

[0073] The kit provided by the invention comprises a delivery device having at least one hollow microneedle designed to intradermally deliver a substance to a depth between .025 and 2 mm which is adapted so that the microneedle is or can be placed in fluid connection with a reservoir adapted for containing a dosage of a vaccine or gene therapeutic. In a preferred embodiment, the kit also contains an effective dosage of a vaccine or gene therapeutic, optionally contained in a reservoir that is an integral part of, or is capable of being functionally attached to, the delivery device. The hollow microneedle is preferably between about 31 to 50 gauge, and may be part of an array of, for example, 3-6 microneedles.

[0074] In a particularly preferred embodiment, the kit of the invention comprises a hub portion being attachable to the prefillable reservoir storing the vaccine; at least one microneedle supported by said hub portion and having a forward tip extending away from said hub portion; and a limiter portion surrounding said microneedle(s) and extending away from said hub portion toward said forward tip of said microneedle(s), said limiter including a generally flat skin engaging surface extending in a plane generally perpendicular to an axis of said microneedle(s) and adapted to be received against the skin of a mammal to administer an intradermal injection of the vaccine, said microneedle(s) forward tip(s) extending beyond said skin engaging surface a distance approximately 0.5 mm to 2.0 mm wherein said limiter portion limits penetration of the microneedle(s) into the dermal layer of skin of the mammal.

[0075] To use a kit as envisioned by the instant invention the practitioner would break a hermetic seal to provide access to the microdevice and optionally, the vaccine or immunogenic or therapeutic composition. The composition may be preloaded within the microdevice in any form including but not limited to gel, paste, oil, emulsion, particle, nanoparticle, microparticle, suspension or liquid. The composition may be separately packaged within the kit package, for example, in a reservoir, vial, tube, blister, pouch or the like. One or more of the constituents of the formulation may be lyophilized, freeze-dried, spray freeze-dried, or in any other reconstitutable form. Various reconstitution media, cleansing or disinfective agents, or topical sterilizants (alcohol wipes, iodine) can further be provided if desired. The practitioner would then load or integrate the substance if necessary into the device and then administer the formulation to the patient using the ID injection microdevice.

[0076] In a specific embodiment, the invention comprises kits comprising a device for intradermal delivery and a chimeric flavivirus vaccine formulation of the invention as described herein. In another specific embodiment, the invention provides a kit for use in inducing an immune response to a flavivirus viral antigen in a subject, said kit comprising: (a) a chimeric yellow fever virus expressing an envelope gene product of a flavivirus *e.g.*, Dengue, JE and (b) a device that targets the intradermal compartment of the subject's skin.

6. EXAMPLES

[0077] Having described the invention in general, the following specific but not limiting examples and reference to the accompanying Figures set forth various examples for practicing the invention.

[0078] A representative example of dermal-access microdevice (MDD device) comprising a single needle were prepared from 34 gauge steel stock (MicroGroup, Inc., Medway, MA) and a single 28° bevel was ground using an 800 grit carborundum grinding wheel. Needles were cleaned by sequential sonication in acetone and distilled water, and flow-checked with distilled water. Microneedles were secured into small gauge catheter tubing (Maersk Medical) using UV-cured epoxy resin. Needle length was set using a mechanical indexing plate, with the hub of the catheter tubing acting as a depth-limiting control and was confirmed by optical microscopy. The exposed needle length was adjusted to 1 mm using an indexing plate. Connection to the syringe was via an integral Luer adapter at the catheter inlet. During injection, needles were inserted perpendicular to the skin surface, and were held in place by gentle hand pressure for bolus delivery. Devices were checked for function and fluid flow both immediately prior to and post injection. A 30/31 gauge intradermal needle device with 1.5mm exposed length controlled by a depth limiting hub as described in EP 1 092 444 A1 was also used in some Examples.

[0079] *Example 1: ID delivery and expression of model genetic therapeutic/prophylactic agents, guinea pig model.*

[0080] Uptake and expression of DNA by cells *in vivo* are critical to effective gene therapy and genetic immunization. Plasmid DNA encoding the reporter gene, firefly luciferase, was used as a model gene therapeutic agent (Aldevron, Fargo, ND). DNA was administered to Hartley guinea pigs (Charles River, Raleigh, NC) intradermally (ID) via the Mantoux (ID-Mantoux) technique using a standard 30G needle or was delivered ID via MDD (ID-MDD) using a 34G steel micro-cannula of 1mm length (MDD device) inserted approximately perpendicular. Plasmid DNA was applied topically to shaved skin as a negative control (the size of the plasmid is too large to allow for passive uptake into the skin). Total dose was 100 µg per animal in

total volume of 40 μ l PBS delivered as a rapid bolus injection (<1 min) using a 1cc syringe. Full thickness skin biopsies of the administration sites were collected 24 hr. following delivery, were homogenized and further processed for luciferase activity using a commercial assay (Promega, Madison, WI). Luciferase activity was normalized for total protein content in the tissue specimens as determined by BCA assay (Pierce, Rockford, IL) and is expressed as Relative Light Units (RLU) per mg of total protein (n=3 animals per group for Mantoux and Negative control and n=6 for MDD device).

[0081] The results (Figure 1) demonstrate strong luciferase expression in both ID injection groups. Mean luciferase activity in the MDD and Mantoux groups were 240- and 220-times above negative controls, respectively. Luciferase expression levels in topical controls were not significantly greater than in untreated skin sites (data not shown). These results demonstrate that the method of the present invention using MDD devices is at least as effective as the Mantoux technique in delivering genetic materials to the ID tissue and results in significant levels of localized gene expression by skin cells in vivo.

Example 2: *ID delivery and expression of model genetic therapeutic/prophylactic agents, rat model.*

[0082] Experiments similar (without Mantoux control) to those described in Example 1 above were performed in Brown-Norway rats (Charles River, Raleigh, NC) to evaluate the utility of this platform across multiple species. The same protocol was used as in Example 1, except that the total plasmid DNA load was reduced to 50 μ g in 50 μ l volume of PBS. In addition, an unrelated plasmid DNA (encoding b-galactosidase) injected ID (using the MDD device) was used as negative control. (n=4 animals per group). Luciferase activity in skin was determined as described in Example 1 above.

[0083] The results, shown in Figure 2, demonstrate very significant geneexpression following ID delivery via the MDD device. Luciferase activity in recovered skin sites was > 3000-fold greater than in negative controls. These results

further demonstrate the utility of the method of the present invention in delivering gene based entities *in vivo*, resulting in high levels of gene expression by skin cells.

Example 3: *ID delivery and expression of model genetic therapeutic/prophylactic agents, pig model.*

[0084] The pig has long been recognized as a preferred animal model for skin based delivery studies. Swine skin is more similar to human skin in total thickness and hair follicle density than is rodent skin. Thus, the pig model (Yorkshire swine; Archer Farms, Belcamp, MD) was used as a means to predict the utility of this system in humans. Experiments were performed as above in Examples 1 and 2, except using a different reporter gene system, β -galactosidase (Aldevron, Fargo, ND). Total delivery dose was 50 μ g in 50 μ l volume. DNA was injected using the following methods I) via Mantoux method using a 30G needle and syringe, ii) by ID delivery via perpendicular insertion into skin using a 30/31G needle equipped with a feature to limit the needle penetration depth to 1.5mm, and iii) by ID delivery via perpendicular insertion into skin using a 34G needle equipped with a feature to limit the needle penetration depth to 1.0mm (MDD device). The negative control group consisted of ID delivery by i-iii of an unrelated plasmid DNA encoding firefly luciferase. (n=11 skin sites from 4 pigs for the ID Mantoux group; n=11 skin sites from 4 pigs for ID, 30/31G, 1.5mm device; n=10 skin sites from 4 pigs for ID, 34G, 1mm device; n=19 skin sites from 4 pigs for negative control.) For the negative control, data from all 3 ID delivery methods were combined since all 3 methods generated comparable results.

[0085] Reporter gene activity in tissue was determined essentially as described in Example 1, except substituting the β -galactosidase detection assay (Applied Biosystems, Foster City, CA) in place of the luciferase assay.

[0086] The results, shown in Figure 3, indicate strong reporter gene expression in skin following all 3 types of ID delivery. Responses in the ID-Mantoux group were 100-fold above background, compared to a 300-fold increase above background in the ID, 34G, 1mm (MDD) group and 20-fold increase above

background in the ID, 30G, 1.5mm (30 g, 1.5mm) group. Total reporter gene expression by skin cells, as measured by reporter gene mean activity recovered from excised skin tissue biopsies, was strongest in the ID, 34G, 1mm (MDD) group at 563,523 RLU/mg compared to 200,788 RLU/mg in the ID, 30G Mantoux group, 42,470 RLU/mg in the ID (30G, 1.5mm) group and 1,869 RLU/mg in the negative controls. Thus, ID delivery via perpendicular insertion of a 34G, 1.0mm needle (MDD) results in superior uptake and expression of DNA by skin cells as compared to the standard Mantoux style injection or a similar perpendicular needle insertion and delivery using a longer (1.5mm), wider diameter (30G) needle. Similar studies using these 3 devices and methods to deliver visible dyes also demonstrate that the 34G, 1.0mm needle results in more consistent delivery to the ID tissue than the other 2 needles/methods and results in less “spill-over” of the administered dose into the subcutaneous (SC) tissue.

[0087] These differences were unexpected since all 3 devices and methods theoretically target the same tissue space. However, it is much more difficult to control the depth of delivery using a lateral insertion (Mantoux) technique as compared to a substantially perpendicular insertion technique that is achieved by controlling the length of the cannula via the depth-limiting hub. Further, the depth of needle insertion and exposed height of the needle outlet are important features associated with reproducible ID delivery without SC “spill-over” or leakage on the skin surface.

[0088] These results further demonstrate the utility of the methods of the present invention in delivering gene based entities in larger mammals *in vivo*, resulting in high levels of gene expression by skin cells. In addition, the similarities in skin composition between pigs and humans indicate that comparable clinical improvements should be obtained in humans.

Example 4: *Indirect measurement of localized tissue damage following ID delivery*

[0089] Results presented in Example 3 above suggest that there may be unexpected improvements in efficacy attained by MDD-based ID delivery

compared to that attained by Mantoux-based injections using standard needles. In addition, the MDD cannula mechanically disrupt a smaller total area of tissue since they are inserted to a reduced depth compared to standard needles and are not laterally “snaked” through the ID tissue like Mantoux-style injections. Tissue damage and inflammation leads to the release of several inflammatory proteins, chemokines, cytokines and other mediators of inflammation.

[0090] Thus, total protein content at recovered skin sites can be used as an indirect measurement of tissue damage and localized inflammation induced by the two delivery methods. Total protein levels were measured in recovered skin biopsies from pig samples presented in Example 3 above (excluding the 30g, 1.5mm) using a BCA assay (Pierce, Rockford, IL). Both methods of delivery induced an increase in total protein content compared to untreated skin, as shown in Figure 4. However, total protein levels in recovered skin biopsies from the ID Mantoux group were significantly greater ($p=0.01$ by t-test) than the corresponding levels in the MDD group (2.4 mg/ml vs. 1.5 mg/ml). These results provide indirect evidence to strongly suggest that delivery by the methods of the present invention induces less mechanical damage to the tissue than the corresponding damage induced by Mantoux-style ID injection.

Example 5: *Induction of immune response to influenza DNA vaccine following ID delivery in rats*

[0091] The examples presented above demonstrate that narrow gauge microcannula can be used to effectively deliver model nucleic acid based compounds into the skin resulting in high levels of gene expression by skin cells. To investigate the utility of delivering DNA vaccines by the methods of the present invention, rats were immunized with plasmid DNA encoding influenza virus hemagglutinin (HA) from strain A/PR/8/34 (plasmid provided by Dr. Harriet Robinson, Emory University School of Medicine, Atlanta, GA). Brown-Norway rats ($n=3$ per group) were immunized three times (days 0, 21 and 42) with plasmid DNA in PBS solution (50 μ g per rat in 50 μ l volume delivered by rapid bolus injection) ID using the MDD device as described in Example 2 or IM into the quadriceps using a conventional 30G needle

and 1cc syringe. As a negative control, DNA was applied topically to untreated skin. Sera were collected at weeks 3, 5, 8 and 11 and analyzed for the presence of influenza-specific antibodies by ELISA. Briefly, microtiter wells (Nalge Nunc, Rochester, NY) were coated with 0.1µg of whole inactivated influenza virus (A/PR/8/34; Charles River SPAFAS, North Franklin, CT) overnight at 4°C. After blocking for 1hr at 37 °C in PBS plus 5% skim milk, plates were incubated with serial dilutions of test sera for 1 hr at 37 °C. Plates were then washed and further incubated with horse radish peroxidase conjugated anti-rat IgG, H+L chain (Southern Biotech, Birmingham, AL) for 30 min at 37 °C and were then developed using TMB substrate (Sigma, St. Louis, MO). Absorbance measurements (A_{450}) were read on a Tecan SunriseTM plate reader (Tecan, RTP, NC).

[0092] The results (Figure 5) demonstrate that delivery by the method of the present invention of a genetic influenza vaccine in the absence of added adjuvant induces a potent influenza-specific serum antibody response. The magnitude of this response was comparable to that induced via IM injection at all measured timepoints. No detectable responses were observed in the topical controls. Thus delivery of genetic vaccine by the method of the present invention induces immune responses that are at least as strong as those induced by the conventional route of IM injection.

[0093] To further investigate delivery by the method of the present invention of adjuvanted genetic vaccines, the above described influenza HA-encoding plasmid DNA was prepared using the MPL + TDM Ribi adjuvant system (RIBI Immunochemicals, Hamilton, MT) according to the manufacturer's instructions. Rats (n=3 per group) were immunized and analyzed for influenza-specific serum antibody as described above. Titers in the ID delivery group were comparable to IM following the first and second immunization (week 3-5; Figure 6). After the third dose, however, ID-induced titers were significantly greater ($p=0.03$ by t-test) than the corresponding titers induced via IM injection (Figure 6). At week 11, the mean ID-induced titer was 42,000 compared to only 4,600 attained by IM injection. Topical controls failed to generate an influenza-specific response. Thus, delivery by the method of the present invention of genetic vaccines in the presence of adjuvant

induces immune responses that are stronger than those induced by the conventional route of IM injection.

Example 6: *Induction of immune response to influenza DNA/virus “prime-boost” following ID delivery in rats*

[0094] A recently developed vaccination approach for numerous diseases, including HIV, is the so-called “prime-boost” approach wherein the initial “priming” immunizations and secondary “boosters” employ different vaccine classes (Immunology Today, Apr 21(4): 163-165, 2000). For example, one may prime with a plasmid DNA version of the vaccine followed by a subsequent boost with a subunit protein, inactivated virus or vectored DNA preparation. To investigate delivery by the method of the present invention of these types of vaccination methods, the first experiment of Example 5 was continued for an additional 6 weeks. At week 11, DNA-primed rats were boosted with whole inactivated influenza virus (A/PR/8/34, 100µg in 50µl volume of PBS by rapid bolus injection). Virus was obtained from Charles River SPAFAS, North Franklin, CT. Influenza-specific serum antibody titers were measured at weeks 13 and 17 by ELISA as described above. Both ID delivery and IM injection induced a potent booster effect (Figure 7). Week 17 mean influenza-specific titers were equivalent (titer = 540,000) by both methods of delivery and were significantly greater than the very weak titers observed following unassisted topical delivery (titer = 3200). Thus, delivery by the method of the present invention is suitable for “prime-boost” immunization regimens, inducing immune responses that are at least as strong as those induced by the conventional route of IM injection.

[0095] To evaluate the effect of adjuvant on the “prime-boost” response, the second experiment described in Example 5 was continued for an additional 6 weeks. At week 11, DNA-primed rats were boosted with whole inactivated influenza virus (A/PR/8/34, 100µg in 50µl volume by rapid bolus injection as above). Influenza-specific serum antibody titers were measured at weeks 13 and 17 by ELISA as described above. Both ID delivery and IM injection induced a potent booster effect (Figure 8). Mean titers in the ID delivery group were greater than via IM injection following the virus boost; at week 13, the ID-MDD(MDD)

mean titer was 540,000 compared to 240,000 by IM injection and 3,200 by unassisted topical application. Thus, delivery by the method of the present invention is suitable for “prime-boost” immunization regimens incorporating adjuvants, inducing immune responses that are stronger than those induced by the conventional route of IM injection.

Example 7: *Induction of immune response to influenza virus vaccine following ID delivery in rats*

[0096] To investigate the utility of delivering conventional vaccines by the method of the present invention in, rats were immunized with an inactivated influenza virus preparation as described in Example 6 via ID delivery or intramuscular (IM) injection with a standard needle and syringe. As negative control, vaccine solution was applied topically to untreated skin; the large molecular weight of the inactivated influenza virus precludes effective immunization via passive topical absorption. As above, vaccine dose was 100 µg total protein in 50 µl volume per animal delivered by rapid bolus injection (< 1 min). Rats were immunized 3 times (days 0, 21 and 42); serum was collected and analyzed for influenza-specific antibodies by ELISA as above on days 21, 35 and 56; n=4 rats per group.

[0097] The results, shown in Figure 9, indicate that ID delivery induces potent antigen specific immune responses. Similar levels of antibody were induced by the 2 injection routes, IM and ID. Peak geometric mean titers were somewhat higher in the ID-MDD group (MDD); 147,200 compared to 102,400 via IM injection. Topical application of the vaccine stimulated only very weak responses (peak mean titer = 500). Thus, ID delivery of conventional vaccines at high doses induces immune responses that are at least as strong as those induced by the conventional route of IM injection.

Example 8: *Induction of immune response to influenza vaccine following ID delivery via in pigs*

[0098] As noted above, the pig represents an attractive skin model that closely mimics human skin. To test ID delivery devices in vaccine delivery, Yorkshire swine were immunized with an inactivated influenza vaccine as above, comparing ID delivery ID with IM injection . Pigs were immunized on days 0, 21 and 49; serum was collected and analyzed for influenza-specific antibodies by ELISA as above on days 14, 36, 49 and 60. Pig-specific secondary antibodies were obtained from Bethyl Laboratories, Montgomery, TX..

[0099] The results (Figure 10) indicate that ID delivery induces potent antigen specific immune responses. Similar levels of antibody were induced by the 2 injection routes, IM and ID. Peak geometric mean titers were slightly higher in the MDD group; 1,333 compared to 667 via IM injection. Thus, ID delivery of conventional vaccines at high doses induces immune responses that are at least as strong as those induced by the conventional route of IM injection.

Example 9: ID delivery of Lower doses of influenza vaccine

[00100] In Example 7, rats were immunized with 100 μ g of inactivated influenza virus via ID injection, or IM using a conventional needle and syringe. At such a high dose, both delivery methods induced similar levels of serum antibodies, although at the last time-point the ID-induced titer was slightly greater than that induced by IM.

[00101] To further study the relationship between method of delivery and dosage level, rats were immunized with reduced doses of inactivated influenza virus, ranging from 1 μ g to 0.01 μ g per animal, using the ID and IM routes of administration as above. Rats were given 3 immunizations (days 0, 21 and 42) and were analyzed for serum anti-influenza antibodies at days 21, 35 and 56 (n=4 rats per group). Data shown in Figure 11 reflect titers at day 56, although similar trends were observed at day 21 and day 35. ID delivery (MDD) resulted in a significant antibody response that did not differ significantly in magnitude at the 3 doses tested; *i.e.*, delivery of as little as 0.01 μ g (10ng) induced comparable titers to those observed using 100-fold more vaccine (1 μ g). In contrast, a significant reduction in titer was

observed when the IM dose was reduced from 1 μ g to 0.1 μ g and again when the dose was further reduced to 0.01 μ g. In addition, there was considerably less variability in the titers induced via ID delivery as compared to IM. Notably, no visible side reactions (adverse skin effects) were observed at the ID administration sites.

[00102] The results strongly indicate that ID delivery by the method of the present invention enables a significant (at least 100-fold) reduction in vaccine dose as compared to IM injection. Significant immune responses were observed using nanogram quantities of vaccine. Similar benefits would be expected in clinical settings.

[00103] The results described herein demonstrate that ID injection of vaccine and genetic material can be reproducibly accomplished the methods of the present invention. This method of delivery is easier to accomplish than standard Mantoux-style injections or IM and, in one embodiment, by virtue of its limited and controlled depth of penetration into the skin, is less invasive and painful. In addition, this method provides more reproducible ID delivery than via Mantoux style injections using conventional needles resulting in improved targeting of skin cells with resultant benefits as described above.

[00104] In addition, the method is compatible with whole-inactivated virus vaccine and with DNA plasmids without any associated reduction in biological potency as would occur if the virus particles or plasmid DNA were sheared or degraded when passed through the microcannula at the relatively high pressures associated with ID delivery in vivo. The results detailed herein demonstrate that stronger immune responses are induced via ID delivery, especially at reduced vaccine doses, thus potentially enabling significant dose reductions and combination vaccines in humans.

[00105] The results presented above show improved immunization via ID delivery using devices such as those described above as compared to standard intramuscular (IM) injection using a conventional needle and syringe. The dose reduction study (Example 9), shows that ID delivery induces serum

antibody responses to an influenza vaccine in rats using at least 100-fold less vaccine than required via IM injection. If applicable in a clinical setting, such dose reduction would reduce or eliminate the problem of influenza vaccine shortages common across the world. In addition, such dose reduction capabilities may enable the delivery of a greater number of vaccine antigens in a single dose, thus enabling combination vaccines. This approach is of particular relevance to HIV vaccines where it likely will be necessary to immunize simultaneously with several genetic variants / sub-strains in order to induce protective immunity.

[00106] Similar benefits are expected with other types of prophylactic and therapeutic vaccines, immuno-therapeutics and cell-based entities by virtue of the improved targeting of the immune system using the methods and devices of the present invention.

[00107] In another embodiment, it is within the scope of the present invention to combine the ID delivery of the present invention with convention methods of administration, for example IP, IM, intranasal or other mucosal route, or SQ injection, topical, or skin abrasion methods to provide improvement in immunological or therapeutic response. This would include for example, vaccines and or therapeutics of the same or different class, and administration simultaneously or sequentially.

[00108] All references cited in this specification are hereby incorporated by reference. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art relevant to patentability. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

[00109] The embodiments illustrated and discussed in the present specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention, and should not be considered as limiting the scope of the present invention. The exemplified embodiments of the invention may be modified or varied, and elements added or omitted, without

departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is therefore to be understood that, within the scope of the claims and their equivalents, the invention may be practiced otherwise than as specifically described.